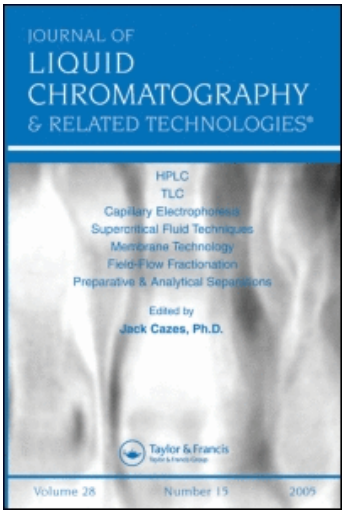

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Predictive Optimization of Gradient-Elution Liquid Chromatography

P. Jandera[a]
[a] University of Chemical Technology Leninovo, Pardubice, Czechoslovakia

To cite this Article Jandera, P.(1989) 'Predictive Optimization of Gradient-Elution Liquid Chromatography', Journal of Liquid Chromatography & Related Technologies, 12: 1, 117 — 137
To link to this Article: DOI: 10.1080/01483918908049193
URL: http://dx.doi.org/10.1080/01483918908049193

JOURNAL OF LIQUID CHROMATOGRAPHY, 12(1&2), 117-137 (1989)

PREDICTIVE OPTIMIZATION OF GRADIENT-ELUTION LIQUID CHROMATOGRAPHY

P. JANDERA
University of Chemical Technology
Leninovo nám 565
532 10 Pardubice, Czechoslovakia

ABSTRACT

The methods for calculation of retention volumes and bandwidths in liquid chromatography with binary gradients, ternary "solvent-strength" gradients, ternary "selectivity" gradients and "combined selectivity - solvent strength" ternary gradients are surveyed. These calculation methods form the basis for the predictive optimization of profiles of binary and ternary gradients. Various strategies for predictive optimization are discussed and illustrated by practical optimization example of the gradient-elution separation of a mixture of mono- and di- aminoanthraquinones.

INTRODUCTION

The optimization of the mobile phase composition in HPLC with isocratic elution or of the gradient profile in gradient-elution HPLC may use either the imperfect empirical "trial-and-error" approach or some of the rational systematic approaches. These systema-

tic optimization methods have been reviewed recently by Schoenmakers[1]. In addition to statistical sequential and simultaneous optimization methods, which do not require any preliminary information on the retention mechanism, but usually require a number of scouting experimental runs in the chromatographic system to be optimized, predictive and interpretive optimization methods may be used. The predictive methods rely on some theoretical models of the retention mechanism in a given chromatographic system, which allow to postulate quantitative mathematical relationships between the optimized parameters of the chromatographic separation and on an adequate criterion of the quality of separation, such as resolution or some sum criterion[1].

Most of the optimization effort has been devoted to HPLC with isocratic elution. However the advantages of gradient elution for separation of sample mixtures with a wide retention range are appreciated now and gradient elution is becoming a routine tool in analytical laboratories[2]. Kirkland and Glajch[3] described the application of their "overlapping resolution mapping" procedure to the optimization of reversed-phase gradient elution. Recently, Dolan et al.[4] have introduced so-called "Dry Lab G" optimization method, which is based on computer simulation of reversed-phase gradient-elution separations. Earlier, we have suggested several predictive optimization procedures for binary, ternary and stepwise gradient-elution chromatography in reversed-phase, ion-exchange and straight-phase systems[5-9].

It was the objective of this work to provide a systematic survey and comparison of our optimization procedures for gradient-elution HPLC and to elucidate some possible problems on practical examples.

ISOCRATIC-ELUTION CHROMATOGRAPHY

Our predictive procedures for optimization of gradient-elution separations make use of the dependence of the solute capacity factors, k', on the concentration /φ, in volume per cent/ of the more efficient eluting component /or components/ in binary or ternary mobile phases. The mathematical form of this dependence is given by the chromatographic system used and it may be rather complex. However relatively simple equations are suitable for the prediction of retention data and for the optimization purposes in a number of chromatographic systems[6].

In many straight-phase chromatographic systems using polar adsorbents or polar chemically bonded stationary phases and binary mobile phases, the dependence of k' on the concentration φ of a more polar organic solvent in a non-polar one may be described by a simple equation[6,10]:

$$k' = a \cdot \varphi^{-m} \qquad /1/$$

a and m are experimental constants depending on the structure of the solute, on the nature of the stationary phase and the solvents used and on the temperature. The eq./1/ can often be used also in ion-exchange chromatography of completely ionized solutes, but molar concentration, c, of counter-ions in the mobile phase should be used instead of φ[6,11].

In reversed-phase chromatographic systems, similar equation can often be used to describe the retention--mobile phase composition dependence[6,10,12,13]:

$$\log k' = a - m \cdot \varphi \qquad /2/$$

Here, φ is the volume concentration of an organic solvent in aqueous-organic binary mobile phase and the meaning of the constants a, m is similar as in the eq./1/.

The eqs./1/ and /2/ or other similar equations may be used as the basis of predictive optimization of the composition of binary mobile phases, after introduction into the definition equation for the resolution of a pair of solutes 1 and 2 with retention volumes V_{R1}, V_{R2}, capacity factors k_1', k_2' and bandwidths w_1, w_2 on a column with N theoretical plates:

$$R_s = 2\frac{V_{R2} - V_{R1}}{w_1 + w_2} = \frac{\sqrt{N}}{2} \cdot \frac{k_2' - k_1'}{k_1' + k_2' + 2} \qquad /3/$$

The resulting dependence of resolution on mobile phase composition can be used for two different optimization procedures: 1: The $R_s - \varphi$ relationship makes it possible to calculate directly the concentration φ_{opt} necessary to achieve the resolution desired for one or all of the pairs of compounds in the sample mixture[10]. 2: The resolution for all the pairs of sample compounds is calculated in dependence on φ and a "window diagram" is constructed, from which the φ_{opt} is selected that yields the best resolution of all the sample compounds in the shortest time of separation[11].

The $k' - \varphi$ dependencies can be used for the optimization of binary mobile phase gradients in a similar way.

The constants a, m of the eqs./1/ or /2/ should be determined in independent experiments for the solutes analyzed. For this purpose, regression analysis of the experimental data measured at 2 - 5 different mobile phase compositions is the most straightforward approach[14], but the data obtained in two or more gradient-elution experiments with different gradient profiles may also be used for this purpose[4,14].

The use of three- and more- component mobile phases comprised of one weak and two or more stronger eluents has become popular because of the possibility of fine tuning the separation selectivity by adjusting the con-

centration ratio of the stronger eluents in the mobile phase[15,16]. Predictive procedures can be used for optimization of ternary mobile phases, mainly in reversed-phase chromatography, where the mobile phases are comprised of water and two organic solvents x and y /such as methanol, acetonitrile or tetrahydrofuran/ in concentrations φ_x, φ_y. They make use of the following relationship between the solute capacity factors and concentrations φ_x, φ_y[8]:

$$\log k' = \frac{a_x \cdot \varphi_x + a_y \cdot \varphi_y}{\varphi_x + \varphi_y} - m_x \cdot \varphi_x - m_y \cdot \varphi_y \qquad /4/$$

The constants a_x, m_x, a_y, m_y are the constants a, m of the eq./2/ in binary mobile phases containing water and only one organic solvent x or y and are determined in the same way as for the prediction of retention in binary mobile phases. In predictive optimization approaches for ternary mobile phases, the eq./4/ cannot be used for a direct calculation of the optimum concentrations φ_x and φ_y, but these concentrations can be determined from a three-dimensional diagram showing the response surface for all the pairs of sample solutes in dependence on φ_x and φ_y, using the response /resolution/ values calculated from the expression obtained after the introduction of the eq. /4/ into the eq./3/.

GRADIENT-ELUTION CHROMATOGRAPHY

Gradient elution is a powerful tool for improving the separation of sample mixtures with a wide retention range by speeding up the elution of strongly retained compounds. The profile of a binary gradient of increasing concentration of the stronger eluent, φ, in the mobile phase is conveniently characterized by the initial concentration at the start of the gradient, A, by

the slope of the gradient, B, i.e. by the change of φ per volume unit of the eluate and by the shape /curvature/ of the gradient.

Predictive optimization of gradient elution is based on the equations describing the dependence of the retention characteristics, i.e., of the net retention volume, V'_g, and the bandwidth, w_g, in gradient-elution chromatography on the parameters of the gradient. In reversed-phase chromatography, linear gradients are used most frequently. A linear binary gradient of the concentration of an organic solvent in water is described by the equation:

$$\varphi = A + B \cdot V \qquad /5/$$

where V is the volume of the eluate. In the chromatographic systems where the eq./2/ applies, the capacity factor of a sample solute is changed during the elution according to the equation:

$$\log k' = a - m \cdot A - m \cdot B \cdot V \qquad /6/$$

In this case, the theory of gradient elution yields the following expressions for the retention characteristics in dependence on A and B[2,6,10,12]:

$$V'_g = \frac{1}{mB} \cdot \log \left[2.31\ m\ B\ V_M \cdot 10^{(a - mA)} + 1 \right] \qquad /7/$$

$$w_g = \frac{4V_M}{\sqrt{N}} \cdot \left\{ 1 + \left[2.31\ m\ B\ V_M + 10^{(mA - a)} \right]^{-1} \right\} \qquad /8/$$

V_M is the dead volume and N is the number of theoretical plates of the column used; a and m are the experimental parameters of the eq./2/ for the sample solute.

Similar equations were derived also for straight-phase and ion-exchange chromatography, for linear or curved gradient profiles and may be found in refs[2,6,10].

The type of the gradient to be used and the optimization strategy depend on the separation problem. Several different basic situations may be distinguished.

A: If the separation selectivity of all the sample solutes /with a wide retention range/ can be adequately adjusted controlling the concentration ratio of the components of a binary mobile phase, the separation may be improved and the time of separation reduced by using elution with a binary solvent gradient.

B: If the separation selectivity cannot be adequately adjusted in a binary mobile phase, but is suitable at a certain composition of a three-component mobile phase, ternary gradients should be used to improve the separation of sample compounds with a wide retention range. During these gradients, the concentration ratio of the two stronger eluents, φ_x and φ_y, is held constant and the sum $\varphi_T = \varphi_x + \varphi_y$ is increased. Such gradients may be called "ternary solvent-strength gradients".

C: If the separation selectivity for a part of sample solutes is adequate at a certain composition of a ternary mobile phase, but acceptable separation selectivity for the remaining sample compounds can be achieved only at another composition of the ternary mobile phase and the differences between the retention of the individual solutes are not very great, "ternary selectivity gradients" of changing concentration ratio of the two stronger solvents, $g = \varphi_x : \varphi_y$ at a constant sum of the concentrations $\varphi_T = \varphi_x + \varphi_y$ may improve the separation.

D: If the selectivity dependence on mobile phase composition is as in point C, but the sample compounds show a wide retention range, ternary gradients should be used where the concentrations of all the three mobile phase components are changed in such a way that the sum $\varphi_T = \varphi_x + \varphi_y$ is increasing during the elution and the ratio $g = \varphi_x : \varphi_y$ is changed simultaneously. These are "combined selectivity - solvent strength ternary gradients".

E: If the gradients A - D do not provide successful separation, a more efficient column or another type of mobile or stationary phase should be tested.

Predictive Optimization of Binary Gradients

In the predictive optimization methods it is assumed that:

1/ The number of sample solutes of interest is known and the parameters a, m of the eqs./1/ or /2/ have been determined experimentally for each solute.

2/ The column plate number does not depend very significantly on the type of the solute and on the mobile phase composition in the range used for optimization, which usually holds reasonably true with most columns used in the contemporary HPLC.

An ideal chromatogram should show regularly spaced peaks of solutes. This is usually almost impossible to achieve in the elution using continuous gradients and can be sometimes approximated in stepwise gradient elution, the optimization of which is rather complex[7]. In continuous gradient-elution chromatography, the influence of the gradient shape on separation is usually less important than that of the gradient slope, B and of the initial concentration of the efficient eluting component in the mobile phase, A.[6,12] In our optimization procedures it is assumed that an adequate gradient shape /curvature/ has been pre-selected. In reversed-phase chromatography, this is usually a linear gradient. Then, B and A are optimized simultaneously.

1: In the first optimization procedure the gradient parameters A and B are calculated so as to achieve the desired resolution R_{sd} of a "critical" pair of compounds, the separation of which is most difficult. In the same time, the retention volume V'_{gi} of another sample solute, usually the most strongly retained one, should be minimized. The calculation is performed automatically using a computer or a programmable calculator, as follows:

The gradient slope B necessary to achieve $R_s d$ and the corresponding value of V'_{gi} are calculated for zero initial gradient concentration, A = 0, for maximum practically possible $A = A_{max}$ and for $A = 0.5\ A_{max}$, from the appropriate equations for retention volumes and bandwidths, e.g., from the eqs./7/ and /8/. Whichever of the values A = 0 or $A = A_{max}$ yields higher V'_{gi}, it is rejected and the initial interval of the A values is thus halved, either to the limits from 0 to $0.5\ A_{max}$, or from $0.5\ A_{max}$ to A_{max}. The values of B and V'_{gi} are again calculated for the value of A from the middle of the new interval, which is then halved in the same way. This procedure is repeated until the values of A and B corresponding to minimum V'_{gi} are achieved. Further details and an application example of the optimization of gradient-elution reversed-phase separation of a mixture of barbiturates were described elsewhere[5].

2: In the second optimization procedure we select the time of separation, t_G, which determines the volume of the eluate from the start to the end of the gradient elution, V_G, at a constant flow-rate of the mobile phase, F_m : $V_G = t_G \cdot F_m$. By means of a constant value of V_G, the initial concentration of the more efficient eluent, A, and the gradient slope, B are correlated. For a linear gradient controlled by the eq./5/, this correlation is described by the equation:

$$B = \frac{\varphi_G - A}{V_G} \qquad /9/$$

φ_G is the concentration of the more efficient eluent in the mobile phase at the end of the gradient, i.e. at $V = V_G$. The retention volumes V'_g of all sample solutes and resolution R_s of the neighbouring pairs of peaks are calculated for different values A and corresponding B. In reversed-phase chromatography, eqs. /3,7-9/ are used for this purpose. A plot of R_s in de-

pendence on A is constructed for all the solute pairs, in the form of a "window diagram", from which the optimum initial concentration A_{opt} is selected and B_{opt} is calculated from the eq./9/. This optimization procedure was tested on the reversed-phase gradient-elution separation of a mixture of phenylurea herbicides[9].

The first of these two optimization procedures is faster, but it may fail if the sample mixture contains more than two solutes the separation of which is /or may become/ "critical". The second approach provides a "map" of the whole range of the optimized gradient parameters A and B, but it requires a realistic estimate of the separation time before the optimization procedure is started.

Predictive Optimization of Ternary Gradients

In linear ternary gradients, the concentrations φ_x, φ_y of two stronger eluting components in the mobile phase, x and y, are changed simultaneously according to the functions:

$$\varphi_x = A_x + B_x \cdot V \qquad /10/$$

$$\varphi_y = A_y + B_y \cdot V \qquad /11/$$

Such gradients are mainly used in reversed-phase separations.

1: The ternary "solvent-strength" gradients work with a pre-selected concentration ratio of two organic solvents x and y in aqueous mobile phases, $\varphi_x : \varphi_y = g$ and the elution strength is increased by increasing linearly the sum of the concentrations $\varphi_T = \varphi_x + \varphi_y$ in the mobile phase during the gradient run: $\varphi_T = A + B.V$. In this case, the eq./4/ can be written as:

$$\log k' = \frac{a_x \cdot g + a_y}{1 + g} - \frac{m_x \cdot g + m_y}{1 + g} \cdot \varphi_T =$$

$$= a_T - m_T \cdot \varphi_T \qquad /12/$$

The eq./12/ is formally identical with the eq./2/, where $\varphi = \varphi_T$. This means that the eqs./7/ and /8/ for retention volumes and bandwidths in reversed-phase chromatography with binary gradients can be used also for calculations of V'_g and w_g in chromatography with "ternary solvent-strength" gradients. Hence the initial concentration, A, and the slope of the gradient, B, can be optimized using the same predictive procedures as for binary gradients. The ratio g, which controls the separation selectivity by means of the parameters a_T and m_T, should be pre-selected. This can be done in a similar way as the optimization of the concentrations φ_x, φ_y in isocratic-elution chromatography with ternary mobile phases.

2: In ternary "selectivity gradients", the sum of the concentrations of the two stronger eluents x and y in the mobile phase, $\varphi_T = \varphi_x + \varphi_y$, and /approximately/ the elution strength are held constant during the gradient elution, but the ratio $\varphi_x : \varphi_y = g$ is changed with time. To hold φ_T constant, an increase of φ_x should be compensated by an equivalent decrease of φ_y and consequently: $B_x = -B_y = B$; $A_x + A_y = \varphi_T$. The ratio g at the start of the gradient elution is $g_o = A_x : A_y$. In this case, the eqs./10/ and /11/ can be written as:

$$\varphi_x = \frac{g_o}{1 + g_o} \cdot \varphi_T + B \cdot V \qquad /13/$$

$$\varphi_y = \frac{1}{1 + g_o} \cdot \varphi_T - B \cdot V \qquad /14/$$

After the introduction of the eqs./13/ and /14/ into the eq./4/ we obtain the following expression for the dependence of log k' on the volume of the eluate, V, formally identical with the eq./6/ for reversed-phase chromatography with binary gradients:

$$\log k' = \frac{a_x g_o + a_y - (m_x g_o - m_y)\cdot\varphi_T}{1 + g_o} + \left(\frac{a_x - a_y}{\varphi_T} - m_x + m_y\right) B \cdot V \qquad /15/$$

This means that the eqs./7/ and /8/ applying for gradient-elution with binary gradients can be also used for calculations of retention volumes and bandwidths in chromatography with ternary "selectivity gradients".

In this case, the sum of the concentrations, φ_T, can be optimized first with respect to the retention time of the last eluted compounds, using the eq./2/ for isocratic elution with binary mobile phases containing only one of the strong eluents x and y. This retention time determines V_G and if the values of φ_T and V_G are known, the slope $B = B_x = -B_y$ and ratio $g_o = A_x : A_y$ can be optimized simultaneously as in the second optimization procedure for binary gradients /see above/.

3: In "combined selectivity - solvent strength ternary gradients" the concentrations of the two stronger eluents x and y are changed simultaneously according to the eqs./10/ and /11/, but the parameters A_x and A_y, B_x and B_y are not correlated. In this case, the retention volume V'_g of a sample solute can be calculated from the equation similar to the eq./7/[8]:

$$V'_g = \frac{1}{m_x B_x + m_y B_y} \cdot \log\left[2.31 V_M (m_x B_x + m_y B_y) \cdot 10^{(a_G - m_x A_x - m_y A_y)} + 1\right] \qquad /16/$$

where a_G is analogous to the parameters a in eq./2/ and is determined as the mean value of the parameters a_x, a_y, during the gradient elution:

$$a_G = \frac{\left(A_x + B_x \cdot \frac{V_g}{2}\right) \cdot a_x + \left(A_y + B_y \cdot \frac{V_g}{2}\right) \cdot a_y}{A_x + A_y + (B_x + B_y) \cdot \frac{V_g}{2}} \qquad /17/$$

An iteration method should be used for calculation of V'_g from the eqs./16/ and /17/.

These ternary gradients are most difficult to optimize. A gradient of increasing φ_x may be designed first, in order to achieve the separation of the group of more strongly retained compounds in as short a time as possible, using the same procedure as for binary gradients. Then, a gradient of decreasing φ_y from an initial value A_y to zero is optimized by calculating the retention volumes and resolution for the individual sample solutes at different A_y, using the eqs./16/ and /17/. Because the value of V'_G is known from the optimization of the gradient of φ_x and because $\varphi_y = 0$ at $V = V_G$, the slope B_y of the decreasing gradient of φ_y can be calculated directly from the eq./9/ for each A_y. From the dependence of the individual R_s values on A_y, the optimum A_y and B_y values yielding the best resolution for all the solute pairs may be selected.

The prediction of retention volumes and the optimization of reversed-phase ternary gradients water - methanol - acetonitrile were tested on the separation of a mixture of phenolic compounds[8].

Selection of the Gradient Volume, V_G

In the second optimization procedure, the appropriate gradient volume, V_G, in eq./9/ should be preset. In present work, possible influence of the selection of V_G on the results of optimization was investigated.

For this purpose, separation of a mixture of aminoanthraquinones in a reversed-phase system was employed. The sample mixture contained 2,6-diaminoanthraquinone /2,6-DAAQ/, 1,2-diaminoanthraquionone /1,2-DAAQ/, 2-aminoanthraquinone /2-AAQ/, 1-aminoanthraquinone /1-AAQ/ and anthraquinone /AQ/; the column ,4.1 x 300 mm, was packed in the laboratory with Silasorb C18, 10 µm, obtained from Lachema, Brno, Czechoslovakia. 1,4-dioxa-

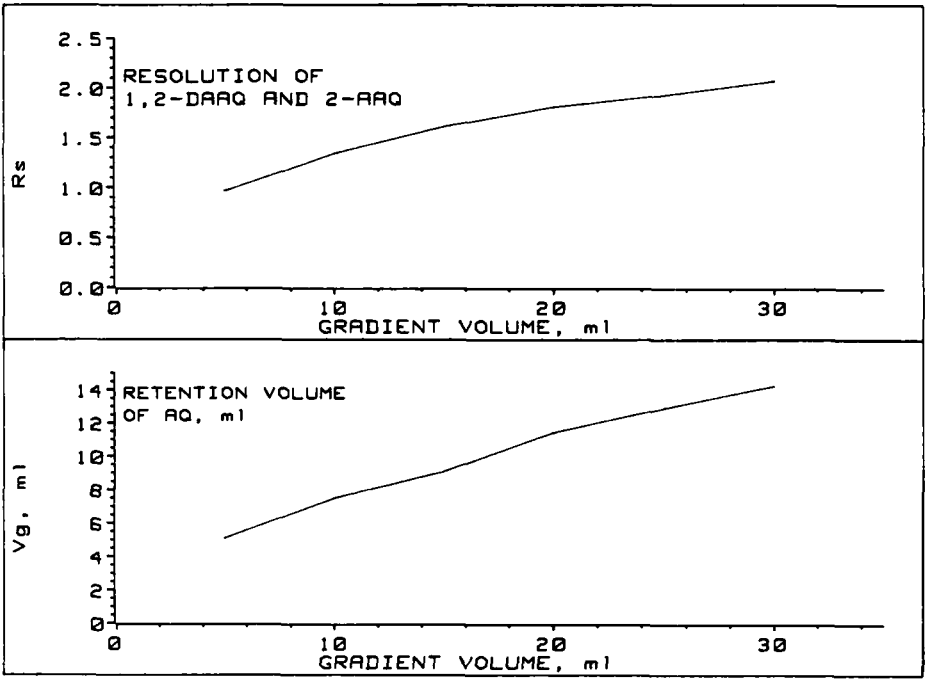

Fig. 1

ne and water were used as the mobile phase components, because it was not possible to achieve good separation of 1,2-DAAQ from 2-AAQ in methanol - water and in acetonitrile - water mobile phases. A 1090M Liquid Chromatograph, Hewlett-Packard, Avondale, USA, equiped with a diode-array detection system, was operated at 254 nm.

With increasing V_G, both the maximum resolution of a given pair of sample compounds and the retention volumes corresponding to the optimized conditions increase. This is illustrated by the dependencies of R_s of 1,2-DAAQ and 2-AAQ and of V_g of anthraquinone on V_G /fig.1/. However, if a certain resolution R_{sd} is desired, the pre-set value of the gradient volume has only minor effect on the separation, as it is demonstra-

TABLE 1

The Results of the Optimization Procedure 2 for Different Pre-set Volumes of the Gradient, V_G.

Column: Silasorb C18, 10 μm, 4.1 x 300 mm. Sample: mixture of 4 aminoanthraquinones /1: 2,6-DAAQ; 2: 1,2-DAAQ; 3: 2-AAQ; 4: 1-AAQ/ and of anthraquinone /AQ//. V_g - elution volume of the last eluted compound, anthraquinone, calculated using eq./7/. A is the initial concentration of 1,4-dioxane in water /in % v/v . 10^{-2}/, and B is the slope of linear gradient /in % v/v . cm^{-3} . 10^{-2}/ calculated using eqs./7-9/ for various desired values of resolution, R_s d, of 1,2-DAAQ and 2-AAQ. N = 3 000; V_M = 2.61; flow-rate: 1 cm^3 min^{-1}.

	V_G /cm^3/	V_g /cm^3/	A	B
R_s d=1.00	10	5.04	0.56	0.044
	15	5.10	0.63	0.025
	20	5.18	0.59	0.021
	25	5.29	0.59	0.017
	30	5.35	0.59	0.014
R_s d=1.25	10	6.19	0.47	0.053
	15	6.23	0.51	0.033
	20	6.16	0.54	0.023
	25	6.29	0.54	0.018
	30	6.25	0.55	0.015
R_s d=1.50	10+	-	-	-
	15	7.59	0.44	0.037
	20	7.48	0.48	0.026
	25	7.54	0.50	0.020
	30	7.56	0.51	0.016
R_s d=1.75	10+	-	-	-
	15+	-	-	-
	20	9.92	0.38	0.031
	25	9.42	0.43	0.023
	30	9.38	0.45	0.018

+ R_s d cannot be achieved

TABLE 2

Calculated /$V_{g,c}$/ and Experimental /$V_{g,e}$/ Values of Elution Volumes of Aminoanthraquinones in Gradient-Elution Chromatography.

Gradient 1: 35 - 100 % 1,4-dioxane in water in 10 min; A = 0.35; B = 0.065. Gradient 2: 40 - 100 % 1,4-dioxane in water in 15 min.; A = 0.4; B = 0.04. Other chromatographic conditions as in Table 1. The gradients were optimized using eqs./7-9/, i.e., prodedure 2, to achieve maximum resolution of 1,2-DAAQ and 2-AAQ in 10 min /1/ and in 15 min /2/. The constants a and m of the eq./2/ used in calculations were obtained by linear regression analysis of the experimental log k' - φ plots. The gradient delay of the instrument /1090M, Hewlett-Packard/ equal to 0.36 ml was added to the V_g values calculated from the eq./7/. R - correlation coefficients. V_g are in cm^3.

compound	a	m	R
2,6-DAAQ	1.406	3.896	0.9993
1,2-DAAQ	2.130	4.540	0.9994
2-AAQ	2,196	4.410	0.9994
1-AAQ	2.572	4.620	0.9997
AQ	2.738	4.484	0.9998

compound	Gradient 1		Gradient 2	
	$V_{g,e}$	$V_{g,c}$	$V_{g,e}$	$V_{g,c}$
2,6-DAAQ	4.58	4.66	4.18	4.38
1,2-DAAQ	5.96	5.87	5.83	5.79
2-AAQ	6.38	6.26	6.40	6.30
1-AAQ	7.32	7.09	7.79	7.51
AQ	8.14	7.87	9.05	8.66

ted by the retention volumes V_g of the last eluted compound, AQ, in the sample mixture /Table 1/. The differences between these values at different pre-set V_G /from 10 to 30 ml/ do not exceed 0.5 ml, even though the values of A and B change with V_G. This means that the retention volume of the last eluted compound is significantly lower than the pre-set gradient time. If the separation is finished when the last compound is

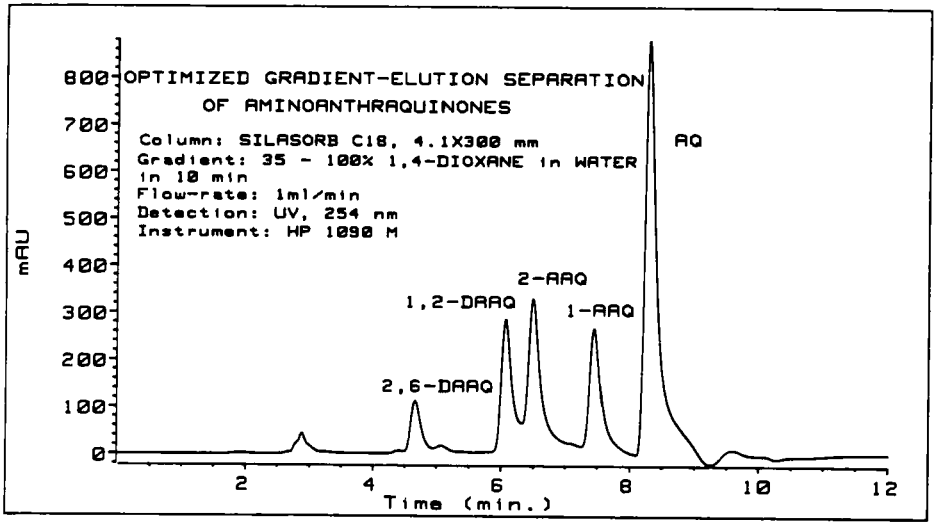

Fig. 2

eluted, the pre-set V_G has not very significant influence on the time of separation.

The precision of the prediction calculations is demonstrated by comparison of the experimental and calculated retention volumes of the individual sample compounds in two gradient-elution chromatography runs optimized so as to achieve maximum resolution of 1,2-DAAQ and 2-AAQ in the gradients with pre-set V_G of 10 and 15 cm^3. The maximum differences between the calculated and predicted retention volumes do not exceed 5% rel., which is acceptable for the optimization purposes /Table 2/. The chromatograms corresponding to these two optimized gradients are shown in figs. 2 and 3. The gradient optimized for the pre-set V_G of 15 min allows to achieve good separation of all the sample compounds in less than 10 minutes, which is considerably shorter a time than that required under isocratic conditions.

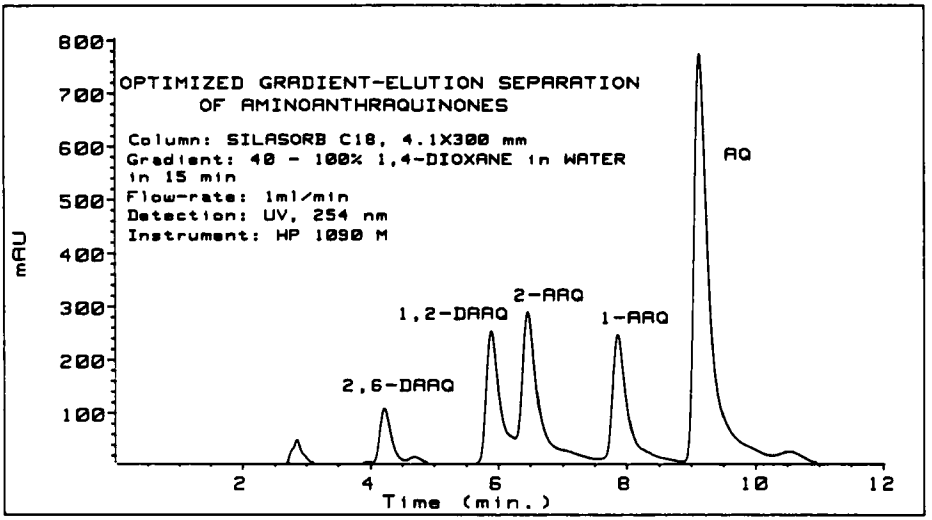

Fig. 3

In conclusion, the second optimization procedure for gradient-elution chromatography is not critically influenced by the pre-set value of the gradient volume, V_G, provided that the elution is finished immediately after the elution of the most strongly retained sample component and that a sufficiently large value of V_G has been pre-set to allow the resolution required for the "critical" pair of compounds to be achieved. If necessary, the optimization procedure may be performed for several pre-set values of V_G and the results compared, but this seems to be rarely necessary in practice.

GLOSSARY OF THE TERMS USED

a - experimental constant in the eqs. /1/ or /2/

a_G - mean value of the constants a_x, a_y in the ternary mobile phase - see eq. /17/

a_T - value of a at the beginning of the ternary gradient - see eq. /12/

a_x, a_y - experimental constants a for the binary mobile phases water - org. solvent x and water - org. solvent y, resp.

$g = \varphi_x : \varphi_y$ - ratio of the concentrations of the more efficient eluting components in the ternary mobile phase

$g_o = A_x : A_y$ - g at the start of the ternary gradient

k' - capacity factor of the solute

m - experimental constant in the eqs. /1/ or /2/

m_T - value of m at the beginning of the ternary gradient - see eq. /12/

m_x, m_y - experimental constants m for the binary mobile phases water - org. solvent x and water - org. solvent y, resp.

t_G - time of the gradient, i.e. the time from the start till the end of the gradient elution, in min.

w_1, w_2 - bandwidths of the solute compounds 1 and 2, resp., in cm^3

w_g - bandwidth of the solute under gradient-elution conditions, in cm^3

A - φ at the beginning of the gradient elution with a linear binary gradient

A_{max} - maximum value of A that can be practically obtained

A_x, A_y - φ_x and φ_y at the beginning of the gradient elution using a linear ternary gradient

B - slope of the linear binary gradient in volume per cents.10^{-2} per 1 cm^3 of the eluate

B_x, B_y - slopes of the changes of φ_x and φ_y, resp., during a linear ternary gradient, in volume per cents.10^{-2} per 1 cm^3 of the eluate

F_m - flow rate of the mobile phase, in $cm^3.min^{-1}$

N - theoretical plate number of the column used

R_s - resolution of the solute compounds 1 and 2

R_{sd} - pre-set resolution that should be achieved in the optimization procedure

V_G - gradient volume, i.e. the volume of the eluate from the start till the end of the gradient elution

V'_g - net retention volume of the solute under gradient-elution conditions

V'_{gi} - V'_g of the sample compound the retention of which should be minimized

V_M - column dead volume, in cm^3

V_{R1}, V_{R2} - retention volumes of the solute compounds 1 and 2, resp., in cm^3

φ - concentration of the more efficient eluting component in the binary mobile phase, in volume per cents.10^{-}

φ_G - φ at the end of the gradient elution

$\varphi_T = \varphi_x + \varphi_y$ - concentration sum of the more efficient eluting components x and y in the ternary mobile phase

φ_x, φ_y - concentrations of the more efficient eluting components x and y, resp., in the ternary mobile phase, in volume per cents.10^{-2}

REFERENCES

1. Schoenmakers, P.J., Optimization of Chromatographic Selectivity, Elsevier, Amsterdam, 1986.
2. Jandera, P. and Churáček, J., Gradient Elution in Column Liquid Chromatography, Elsevier, Amsterdam, 1985.
3. Kirkland, J.J. and Glajch, J.L., J. Chromatogr., 255, 27, 1983.
4. Dolan, J.W., Snyder, L.R. and Quarry, M.A., Chromatographia, 24, 261, 1987.
5. Jandera, P. and Churáček, J., J. Chromatogr., 192, 19, 1980.
6. Jandera, P. and Churáček, J., Advan. Chromatogr., 19, 125, 1980.
7. Jandera, P. and Churáček, J., J. Chromatogr., 170, 1, 1979.
8. Jandera, P., Churáček, J. and Colin, H., J. Chromatogr., 214, 35, 1981.
9. Jandera, P. and Špaček, M., J. Chromatogr., 366, 107, 1986.

10. Jandera, P. and Churáček, J., J. Chromatogr., 91, 207, 1974.

11. Jandera, P., Janderová, M. and Churáček, J., J. Chromatogr., 148, 79, 1978.

12. Jandera, P., Churáček, J. and Svoboda, L., J. Chromatogr., 174, 35, 1979.

13. Snyder, L.R., Dolan, J.W. and Gant, J.R., J. Chromatogr., 165, 3, 1979.

14. Jandera, P. and Churáček, J., J. Chromatogr., 93, 17, 1974.

15. Glajch, J.L., Kirkland, J.J., Squire, K.M. and Minor, J.M., J. Chromatogr., 199, 57, 1980.

16. Glajch, J.L., Kirkland, J.J. and Snyder, L.R., J. Chromatogr., 238, 269, 1982.